Swept confocally-aligned planar excitation (SCAPE) microscopy for high speed volumetric imaging of behaving organisms

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Supplemental Movie Index

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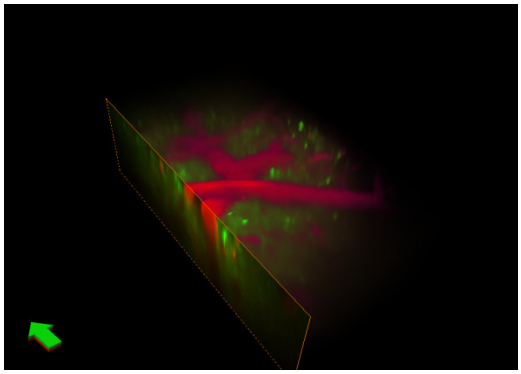
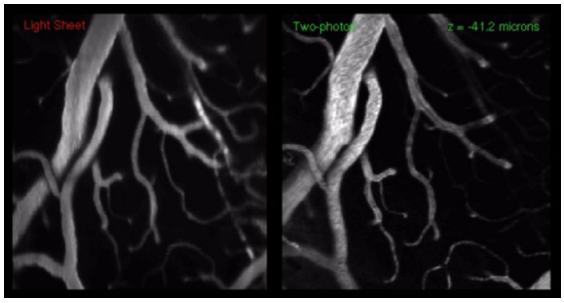
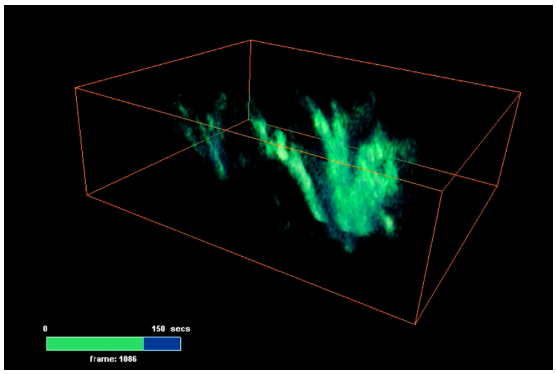


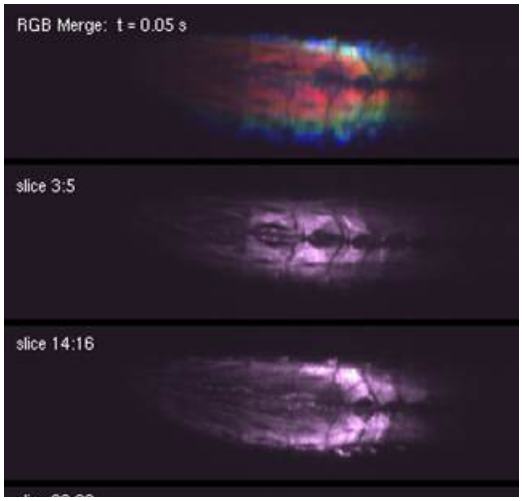
Supplemental Movie 1. 3D volume rendering of SCAPE data acquired in an awake, behaving mouse expressing GCaMP6f in apical dendrites and with Texas red dextran in its vasculature. Movie shows orthogonal slices through a single volume, and then 4D dynamic vascular and neuronal activity at 2x real time. Imaging parameters: 2 color, 350 x 800 x 106 micron volume (100 x 500 x 80 voxels x'-y'-z'), imaged at 10 VPS. See Figure 2 for details.



Supplemental Movie 2. Fly-through depth comparison between SCAPE and two-photon microscopy data acquired on in-vivo mouse brain after intravenous injection of FITC-dx. Imaging parameters SCAPE: 1 color, 480 x 470 x 250 micron volume (cropped, 290 x 315 x 333 voxels x'-y'-z') images at 0.01 VPS. See also Figure 2e and Supplemental Figure 6.

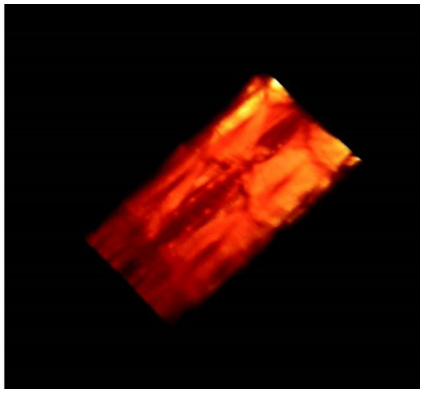


Supplemental Movie 3. 4D dynamic volume rendering of dendritic firing in the cortex of an awake mouse expressing GCaMP5g in apical dendrites, shown as difference data relative to 100 frames after each frame, shown at 2.4 x real-time. Imaging parameters: Single-color, 600 x 650 x 134 micron volumes (240 x 200 x 40 voxels x'-y'-z'), imaged at 10 VPS for 150 seconds. Each pixel time-course was low pass filtered at 1.5Hz to reduce noise. See Figure 3 for details.

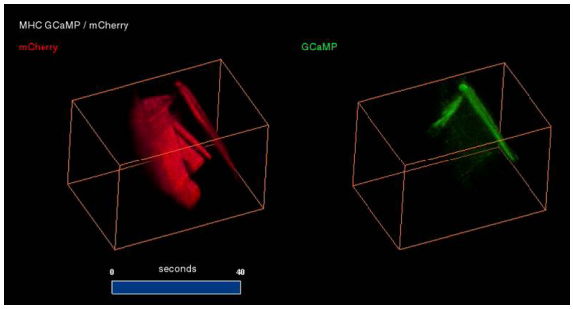


Supplemental Movie 4. Freely moving MHC-GFP, 1st instar *Drosophila* larva, expressing GFP in its body wall muscles, heart tube and gut at 20 VPS. Movie shows 4 different sets of depth slices within SCAPE volume. Top image shows R-G-B color merge of superficial (1-15), central (13:27) and deep (25:39) layers respectively. The imaging stage was translated during image acquisition to re-center the field of view on the larva. Imaging parameters: Single-color, 430 x 1330 x 134 micron volumes (120 x 800 x 80 voxels), imaged at 20 VPS for 40 seconds. See Figure 4 for details.

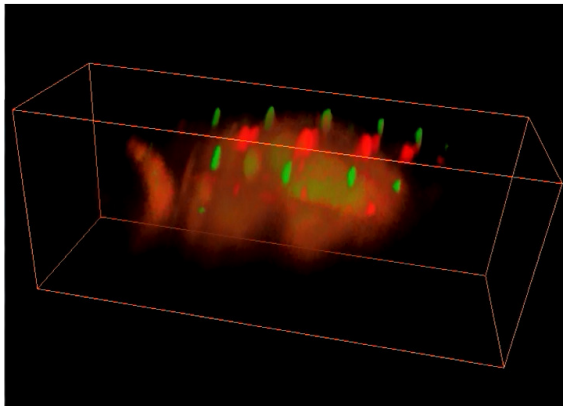
Supplemental Movie 5. 4D volume rendering of a segment of the MHC-GFP *Drosophila* larva dataset shown in Supplemental movie 3 showing heart beat and peristaltic waves in body muscles. A kymograph of this section is shown in Figure 4e.



Supplemental Movie 6. 4D dynamic volume rendering of muscle motion and calcium dynamics in a 3rd instar *Drosophila* larva co-expressing GCaMP6f and mCherry in its muscles. The two color channels are shown side by side, with different views top and bottom. Imaging parameters: 2-color, 300 x 1000 x 264 micron field of view (100 x 300 x 100 voxels x'-y'-z'), imaged at 20 VPS for 40 seconds. Muscle groups exhibit pulses in GCaMP fluorescence as they constrict.



Supplemental Movie 7. 4D dynamic volume rendering of 1st instar larva expressing GFP and tdTomato in different sets of sensory neurons. Food autofluorescence in the gut can also be seen. Imaging parameters: 2-color, 260 x 800 x 265 micron field of view (200 x 650 x 220 voxel, x'-y'-z') acquired at 10 VPS. For 60 seconds.



Supplemental Movie 8. Tracking individual sensory neurons in 4D space (same data set as shown in movie 6). Movie shows top and side maximum intensity projections of raw SCAPE data. Two green neurons were selected in a single frame, and then automatically tracked in 3D space as the animal crawls freely. White crosses in images show neurons, with locations plotted in 3D below.